- Trabajo realizado por el equipo de la Biblioteca Digital de CEU-Universidad San Pablo

- Me comprometo a utilizar esta copia privada sin finalidad lucrativa, para fines de investigación y docencia, de acuerdo con el art. 37 de la M.T.R.L.P.I. (Modificación del Texto Refundido de la Ley de Propiedad Intelectual del 7 julio del 2006)

Zahnd, G.R., J. Steinke, A.E. Renold: Early metabolic affects of human growth hormone. Proc.Soc.Exp.Biol.Med. 105: 455-459 (1960)

Requests for reprints should be addressed to: Dr. Judith S. Stern, Dept. Nutrition, Univ. California, Davis, Calif. 95616 (USA)

Horm. Metab. Res. 8 (1976) 33-37

Effects of 2-Deoxy-D-Glucose, Oligomycin and Theophylline on In Vitro Glycerol Metabolism in Rat Adipose Tissue: Response to Insulin and Epinephrine*

M. Carmen Domínguez and E. Herrera

Cátedra de Fisiología General, Facultad de Biología, Universidad de Barcelona, Barcelona, Spain

Summary

The effects of 2-deoxy-D-glucose (2DG), oligomycin and theophylline on the *in vitro* production and metabolism of glycerol and its response to insulin and epinephrine were studied in epididymal fat pads from fed rats. 2-DG failed to affect basal or epinephrine stimulated glycerol production but it decreased the uptake of 1-^{14}C-glycerol by the tissue and its conversion to glyceride-glycerol. Oligomycin also failed to affect the basal production of glycerol but it inhibited the effect of epinephrine on this parameter as well as the uptake and utilization of 1-^{14}C-glycerol. Theophylline enhanced the production of glycerol by the tissue and this effect was not further augmented by epinephrine. Theophylline also inhibited the uptake and utilization of 1-^{14}C-glycerol; the most pronounced effect of theophylline was observed in the formation of ^{14}C-fatty acids from 1-^{14}C-glycerol in the presence of glucose. Insulin, but not epinephrine, decreased the inhibitory effect of theophylline on glycerol utilization. It is concluded that these compounds affect more intensely the ability of adipose tissue to metabolize glycerol than to release it through lipolysis. The pathway for glycerol utilization in adipose tissue appears to be more sensitive to changes in the availability of ATP than the mechanisms responsible for the release of glycerol fom the tissue.

Key-Words: *Adipose Tissue – Glycerol Utilization – Metabolic Inhibitors – Lipolysis*

Introduction

In preliminary experiments, we have observed that the ability of adipose tissue to metabolize glycerol *in vitro* is influenced by insulin and epinephrine (*Herrera and Domínguez* 1973). Some of the effects of these hormones require the presence of glucose, which could serve to supply a source of ATP for the phosphorylation of glycerol. This suggests that some aspects of glycerol metabolism in adipose tissue might depend on the availability of ATP. One aspect of the present experiments was to determine whether glycerol metabolism is affected by metabolic inhibitors. Thus, 2-deoxy-D-glucose and oligomycin were added to the incubation media and their effects on the production and utilization of glycerol in adipose tissue *in vitro* were noted.

Another aspect of the present study was to investigate whether the elevation in intracellular cyclic AMP produced by epinephrine (*Fain* 1973) could be responsible for the effects of this hormone on glycerol metabolism in adipose tissue. Theophylline was used for this purpose, because it inhibits cyclic AMP phosphodiesterase (*Sutherland and Rall* 1958) in addition to being a metabolic inhibitor for adipose tissue (*Bray* 1966, *Blecher* 1967, *Kupiecki* 1973, *Jarett, Steiner, Smith and Kipnis* 1972). Thus, the effects of theophylline on *in vitro* glycerol metabolism in adipose tissue were determined and compared to the effects of epinephrine.

Material and Methods

Male Wistar rats weighing 179 ± 8 g and fed Purina rat chow *ad libitum* were used. Pieces of epididymal fat pads (22 ± 3 mg) were taken immediately after sacrifice by cervical fracture, and incubated for 180 min at 37°C in Krebs Ringer bicarbonate buffer pH 7.4 (*Umbreit, Burris and Stauffer* 1964), containing 0.5 μCi of 1-^{14}C-glycerol (10 μM) and 10 mg/ml of purified bovine albumin (*Chen* 1967). The incubation procedure has been described in detail previously (*Herrera and Ayanz* 1972). Incubations were carried out in the presence or in the absence of 5 mM 2-deoxy-D-glucose (Sigma Co.), 0.5 μg/ml oligomycin (Sigma Co.), 1 mM theophylline (Sigma Co.), 200 μU/ml pork monocomponent insulin ("Actrapid", from Novo Industries) and 2.8 μM epinephrine bitartrate (Sigma Co.). The media were processed as previously described (*Herrera and Ayanz* 1972) and the enzymatic determinations of glycerol were carried out (*Garland and Randle* 1962). Lipids were extracted from the incubated tissue (*Folch, Lees and Sloane-Stanley* 1957), purified and fractionated as previously described (*Herrera and Lamas* 1970, *Herrera and Ayanz* 1972).

*Supported in part by the Fundación Eugenio Rodríguez Pascual and by a grant from the Presidencia del Gobierno (Comisión Asesora de Investigación Científica y Técnica), Spain.

Received: 28 Nov. 1974 **Accepted:** 6 Oct. 1975

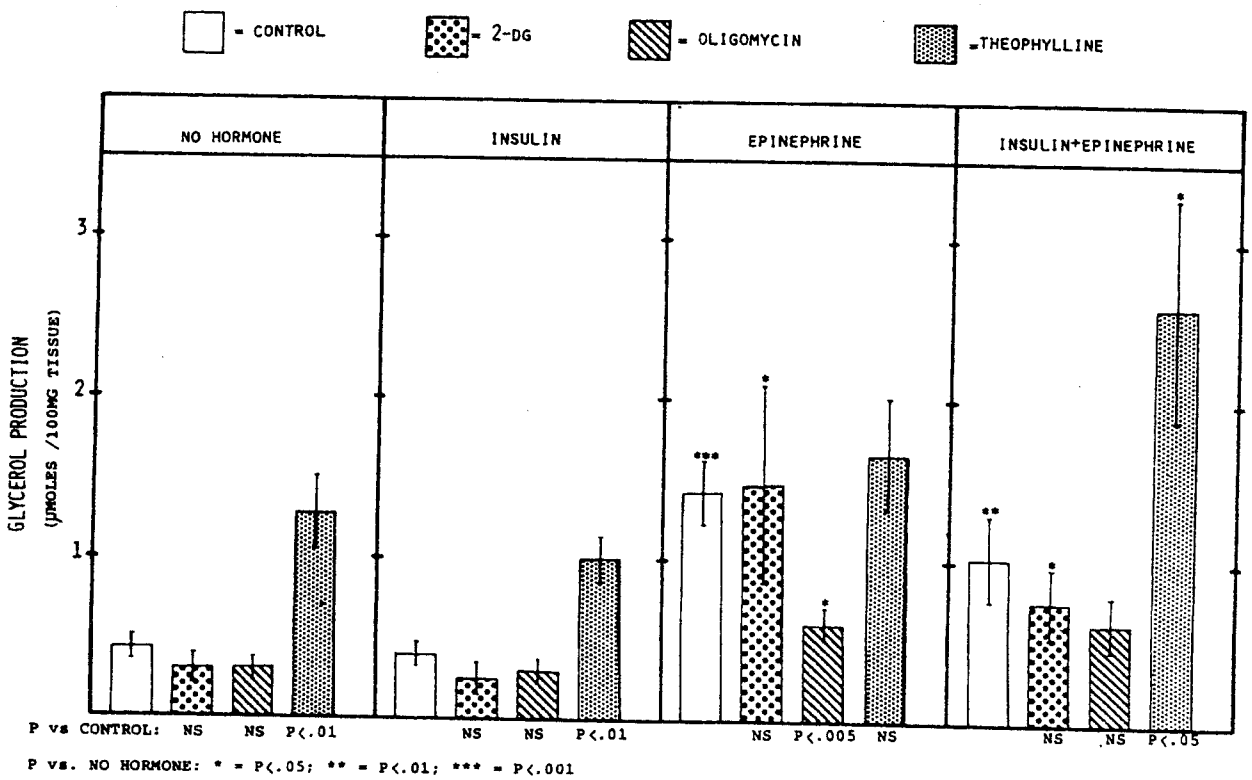

Fig. 1. The effects of 2-DG (5 mM), oligomycin (0.5 µg/ml), theophylline (1 mM), insulin (200 µU/ml) and epinephrine (2.8 µM) on the production of glycerol by pieces of epididymal fat pads incubated "in vitro" in the absence of glucose. Values are means ± SEM of 7 animals/group. p values of the differences vs. the controls are shown at the bottom of the bars (NS = not significant, i.e. $p > 0.05$) and significant differences vs. the tissues incubated in the absence of hormone are denoted by asterisks.

Results

The effects of 2-deoxy-D-glucose (2-DG), oligomycin, theophylline, insulin and epinephrine on the production of glycerol by adipose tissue pieces incubated in the absence of glucose appear in Figure 1. Basal glycerol production (No-Hormone-Control) was unaltered by 2-DG and oligomycin, while theophylline increased it significantly. Insulin failed to affect the production of glycerol compared to the No-Hormone-Control values. Neither 2-DG nor oligomycin affect the production of glycerol in the presence of insulin. Theophylline significantly increased glycerol production in the presence of insulin as well as when no hormone was present in the incubation medium (No-Hormone-Theophylline). Epinephrine stimulated glycerol production significantly compared to the basal level. Oligomycin significantly blocked the increased production of glycerol in the presence of epinephrine. Both 2-DG and theophylline failed to affect the increase in glycerol release produced by epinephrine. Glycerol production was significantly increased from basal levels when both insulin and epinephrine were present in the incubation medium. 2-DG and oligomycin failed to affect glycerol production under these conditions, while theophylline significantly increased it.

Figure 2 shows the effects of the compounds on 1-^{14}C-glycerol uptake. It was significantly decreased by 2-DG, oligomycin and theophylline when compared to the basal level (No-Hormone-Control). Insulin failed to influence significantly the uptake of glycerol, while 2-DG and oligomycin, but not theophylline, significantly inhibited glycerol uptake in the presence of insulin. Epinephrine produced a significant decline in glycerol uptake. Oligomycin and theophylline, but not 2-DG, caused a further significant reduction in glycerol uptake in the presence of epinephrine. The uptake of glycerol was also reduced significantly from basal levels when both insulin and epinephrine were present in the medium. As was the case when epinephrine was present alone, oligomycin and theophylline, but not 2-DG, significantly reduced glycerol uptake in the presence of both hormones.

Table 1 presents the effects of the substances on the *in vitro* metabolism of glycerol in adipose tissue. The formation of labelled CO_2, total lipids and glyceride-glycerol from 1-^{14}C-glycerol was studied using an incubation medium lacking glucose, while the formation of ^{14}C-fatty acids was determined in the presence of 5 mM glucose in the medium, as this metabolite is required to obtain a measurable synthesis of fatty acids from glycerol in adipose tissue (*Dom[illegible]guez and Herrera*, unpublished observations). The in[illegible]oition

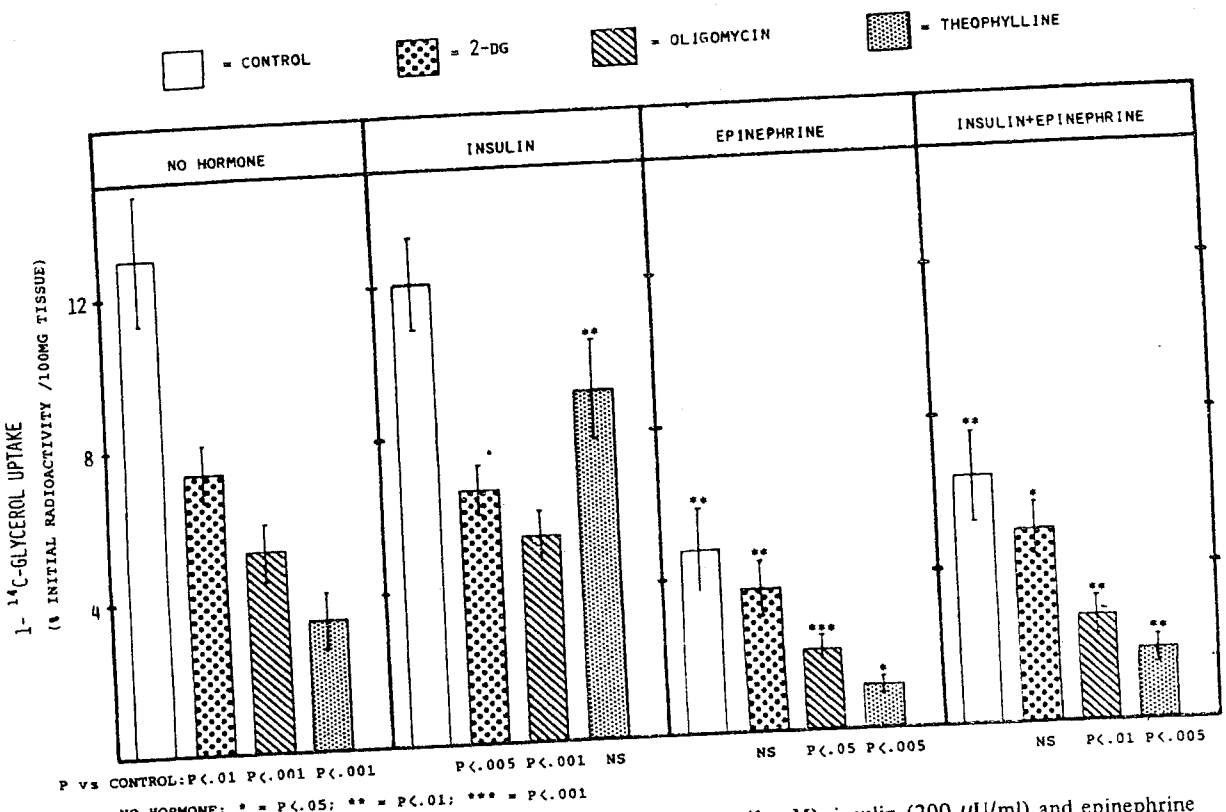

Fig. 2. The effects of 2-DG (5 mM), oligomycin (0.5 μg/ml), theophylline (1 mM), insulin (200 μU/ml) and epinephrine (2.8 μM) on the uptake of labelled glycerol by pieces of epididymal fat pads incubated "in vitro" in the presence of 1-^{14}C-glycerol. No glucose was present in the media. Values are means ± SEM of 7 animals/group. p values of the differences vs. the controls are shown at the bottom of the bars (NS = not significant, i.e. $p > 0.05$) and significant differences vs. the tissues incubated in the absence of hormone are denoted by asterisks.

of glycerol uptake by 2-DG under basal conditions and in the presence of insulin was associated with a significant reduction in the formation of total lipids and glyceride-glycerol. The conversion of 1-^{14}C-glycerol to CO_2 and fatty acids was not significantly altered by 2-DG. A decrease in the formation of $^{14}CO_2$, ^{14}C-total lipids and ^{14}C-fatty acids was observed under all experimental conditions in the presence of oligomycin. Theophylline reduced significantly the formation of all these parameters of glycerol metabolism, except when the incubation medium contained only insulin. Under these conditions the formation of ^{14}C-total lipid and ^{14}C-fatty acids was lowered significantly by theophylline. The formation of $^{14}CO_2$ and ^{14}C-glyceride-glycerol was however unaffected by theophylline in adipose tissue incubated in the presence of insulin. It is to be noted that insulin alone failed to affect significantly the formation of labelled CO_2, total lipids, glyceride-glycerol and fatty acids in the tissue. Epinephrine, like theophylline, reduced significantly the formation of ^{14}C-total lipids, ^{14}C-glyceride-glycerol and ^{14}C-fatty acids. However, the effects of theophylline on these parameters was more pronounced than the epinephrine's. Unlike theophylline, epinephrine failed to affect $^{14}CO_2$ formation significantly. Finally, the effects of insulin and epinephrine together on glycerol metabolism were essentially the same ones as when the incubation medium contained only epinephrine.

Discussion

Taking glycerol production as a measure of "in vitro" lipolysis the present results show that neither the basal lipolytic activity of pieces of epididymal fat pads nor its enhancement by epinephrine were affected by 5 mM 2-DG in the medium. However, this concentration of 2-DG was sufficient to decrease both glycerol uptake and the conversion of glycerol to glyceride-glycerol through a pathway that requires glycerokinase-catalyzed phosphorylation (*Robinson and Newsholme* 1967, *Antony, White and Landau* 1969). 2-DG reduced the cellular content of ATP due to the consumption of ATP by the hexokinase-catalyzed production of 2-deoxy-D-glucose-6-phosphate (*Wick, Drury, Nakada and Wolfe* 1957, *McComb and Yushok* 1964, *Hollenberg and Patten* 1970). It appears therefore, that alterations in ATP levels produce a greater effect on the utilization of glycerol than on the hormone-sensitive lipase. This conclusion is also supported by our results with oligomycin. This compound is an inhibitor of oxidative phosphorylation that causes a marked decrease in ATP levels in adi-

Table 1. The effects of 2-DG oligomycin, theophylline, insulin and epinephrine on the formation of labelled CO_2, total lipids, glyceride-glycerol, and fatty acids from 1-^{14}C glycerol in adipose tissue from rats incubated "in vitro" in the absence or the presence of glucose.

Treatment	Incubation in the absence of glucose			Incubation in the presence of 5 mM glucose
	Formation of $^{14}CO_2$+ (%/100 mg)	Formation of ^{14}C-total lipids (%/100 mg)	Formation of ^{14}C-glyceride-glycerol (%/100 mg)	Formation of ^{14}C-fatty acids (%/100 mg)
No hormone				
Control	2.60 ± 0.49	10.33 ± 1.30	9.63 ± 1.62	2.49 ± 0.61
2-DG	2.60 ± 0.49	4.74 ± 0.52	4.48 ± 0.67	1.55 ± 0.44
p++	N.S.	p < .01	p < .01	N.S.
Oligomycin	0.99 ± 0.38	4.06 ± 0.53	3.53 ± 0.77	1.03 ± 0.15
p	p < .05	p < .01	p < .01	p < .05
Theophylline	1.31 ± 0.32	2.14 ± 0.41	1.79 ± 0.44	0.10 ± 0.03
p	p < .05	p < .001	p < .001	p < .005
Insulin				
Control	2.69 ± 0.60	9.37 ± 0.94	8.07 ± 1.39	2.51 ± 0.55
2-DG	2.54 ± 0.52	4.56 ± 0.58	3.67 ± 0.77	3.10 ± 0.42*
p	N.S.	p < .001	p < .01	N.S.
Oligomycin	1.72 ± 0.39	3.62 ± 0.48	3.10 ± 0.60	1.40 ± 0.27
p	N.S.	p < .001	p < .01	p < .05
Theophylline	1.0 ± 0.16	6.05 ± 1.27**	5.65 ± 1.37**	0.79 ± 0.23**
p	N.S.	p < .05	N.S.	p < .01
Epinephrine				
Control	1.58 ± 0.20	3.97 ± 0.71**	3.59 ± 0.80**	0.47 ± 0.14**
2-DG	1.56 ± 0.40	2.03 ± 0.46**	1.87 ± 0.50**	0.49 ± 0.15*
p	N.S.	p < .05	p < .05	N.S.
Oligomycin	0.74 ± 0.18	1.34 ± 0.21***	1.15 ± 0.27**	0.28 ± 0.12**
p	p < .05	p < .01	p < .01	N.S.
Theophylline	0.53 ± 0.16*	0.58 ± 0.12**	0.54 ± 0.12**	0.11 ± 0.05
p	p < .01	p < .001	p < .01	p < .05
Insulin + Epinephrine				
Control	2.05 ± 0.50	4.38 ± 0.79**	3.60 ± 1.0**	0.97 ± 0.25
2-DG	1.75 ± 0.38	3.18 ± 0.63*	3.12 ± 0.63	0.68 ± 0.29
p	N.S.	N.S.	N.S.	N.S.
Oligomycin	1.0 ± 0.36	1.68 ± 0.33**	1.20 ± 0.30**	0.31 ± 0.05**
p	N.S.	p < .01	p < .05	p < .05
Theophylline	0.93 ± 0.27	0.85 ± 0.23**	0.53 ± 0.06**	0.13 ± 0.03
p	p < .01	p < .001	p < .01	p < .05

(+) Mean ± S.E. of 7 rats/group; %/100 mg = per cent of initial medium radioactivity/100 mg tissue
(++) p-values correspond to the differences of each group vs. the controls (NS = not significant, i.e. p > .05) = p values vs. "No hormone", * = p < .05, ** = p < .01, *** = p < .001.

pose tissue (*Hepp, Challoner and Williams* 1968, *Hollenberg and Patten* 1970). It did not affect basal production of glycerol by adipose tissue. But oligomycin, unlike 2-DG, inhibited the lipolytic effect of epinephrine. It also had a more marked inhibitor effect than 2-DG on the metabolism of glycerol to CO_2 and fatty acids.

Theophylline markedly increased the production of glycerol by adipose tissue and the addition of epinephrine to the medium failed to enhance the production of glycerol above the level obtained in the presence of theophylline alone. This observation confirms the findings of others (*Kuo and DeRenzo* 1969, *Moskowitz and Fain* 1970, *Fain* 1973). The inhibitory effect of theophylline was greater than that of epinephrine on the uptake of labelled glycerol and its conversion to CO_2 and glyceride-glycerol in adipose tissue. The intense effect of theophylline on these parameters is also observed when the dilution of labelled glycerol by the cold glycerol produced during incubation is taken into account (*Herrera and Ayanz* 1972, *Herrera* 1973, *Montoya and Herrera* 1974, *Herrera and Domínguez* 1973). The most pronounced effect of theophylline on glycerol metabolism was the inhibition of fatty acid synthesis in the presence of glucose. The action of theophylline on this parameter was much greater than the effect of epinephrine. An elevation in intracellular cyclic AMP levels can hardly account for the more intense inhibition of fatty acid synthesis by theophylline because cyclic AMP levels are elevated more by epinephrine than by theophylline (*Moskowitz and Fain* 1970, *Fain* 1973). It also is unlikely that the intense inhibitory action of theophylline on fatty acid synthesis from glycerol is due to an effect on the concentration of lipogenetic enzymes, because insulin

partially prevents the action of theophylline on glycerol utilization (Table 1) but does not influence the inhibitory effect of theophylline on protein synthesis (*Jarett, Steiner, Smith and Kipnis* 1972). Theophylline is known to decrease the oxidation of glucose via the pentose shunt pathway (*Kupiecki* 1973). This raises the possibility that the inhibitory action of theophylline on fatty acid synthesis from glycerol might arise from a reduction in the availability of the reduced nucleotides necessary for lipogenesis. Further experimental support is required to sustain this possibility.

In summary, the effects of 2-DG, oligomycin, and theophylline on adipose metabolism and on its response to insulin and epinephrine suggest that the utilization of glycerol is more sensitive to changes in the availability of energy in the tissue than the mechanisms responsible for its release via lipolysis. It must be pointed out, however, that other factors such as compartmentation, substrate composition, intracellular redox potential and intracellular accumulation of free fatty acids might be expected to play a significant role in the modulation of adipose tissue glycerol metabolism in response to these substances.

References

Antony, G., L.W. White, B.R. Landau: Metabolism of D- and L-glyceraldehyde in adipose tissue: a stereochemical probe for glycerokinase activity. J.Lipid Res. 10: 521-527 (1969)

Blecher, M.: Evidence for the involvement of cyclic-3-5-adenosine monophosphate in glucose utilization by isolated rat epididymal adipose cells. Biochem.Biophys.Res.Commun. 27: 560-567 (1967)

Bray, G.A.: Dissociation of glucose oxidation and lipolysis in adipose tissue. Federation Proc. 25: (Abstr.) 271 (1966)

Chen, R.F.: Removal of fatty acids from serum albumin by charcoal treatment. J.biol.Chem. 242: 173-181 (1967)

Fain, J.N.: Biochemical aspects of drug and hormone action on adipose tissue. Pharmacological Reviews 25: 67-118 (1973)

Folch, J.M., M. Lees, G.H. Sloane-Stanley: A simple method for the isolation and purification of total lipids from animal tissues. J.biol.Chem. 226: 497-509 (1957)

Garland, P.B., P.J. Randle: A rapid enzymatic assay for glycerol. Nature 196: 987-988 (1962)

Hepp, D., D.R. Challoner, R.H. Williams: Respiration in isolated fat cells and the effects of epinephrine. J.biol.Chem. 243: 2321-2327 (1968)

Herrera, E.: Effect of albumin on glycerol metabolism in rat adipose tissue. Rev.Esp.Fisiol. 29: 155-162 (1973)

Herrera, E., A. Ayanz: Calculation of lipolysis and esterification from glycerol metabolism in rat adipose tissue. J.Lipid Res. 13: 802-809 (1972)

Herrera, E., M.C. Domínguez: Effects of epinephrine and insulin on glycerol utilization by adipose tissue. VIII Congress of International Diabetes Federation. Excerpta Medica 280: 100 (1973)

Herrera, E., L. Lamas: Utilization of glycerol by rat adipose tissue *in vitro*. Biochem.J. 120: 433-434 (1970)

Hollenberg, C.H., R.L. Patten: Relation of fat cell ATP content to lipolysis induced by dibutyryl 3,5-cyclic AMP. Metabolism 19: 856-864 (1970)

Jarrett, L., A.L. Steiner, R.M. Smith, D.M. Kipnis: The involvement of cyclic AMP in the hormonal regulation of protein synthesis in rat adipocytes. Endocrinology 90: 1277-1284 (1972)

Kuo, J.F., E.C. De Renzo: A comparison of the effects of lipolytic and antilipolytic agents on adenosine 3,5-monophosphate levels in adipose cells as determined by prior labeling with adenine-8-^{14}C. J.biol.Chem. 244: 2252-2260 (1969)

Kupiecki, F.P.: Lipolysis and reesterification: effects of some inhibitors of adenosine 3,5-cyclic monophosphate phosphodiesterase. J.Lipid Res. 14: 250-254 (1973)

McComb, R.B., W.D. Yushok: Metabolism of ascites tumor cells. IV Enzymatic reactions involved in adenosine triphosphate degradation induced by 2-deoxyglucose. Cancer Res. 24: 198-203 (1964)

Montoya, E., E. Herrera: Effect of thyroid status on glycerol metabolism in adipose tissue of fasted male rats. Hormone Res. 5: 129-140 (1974)

Moskowitz, J., J.N. Fain: Stimulation by growth hormone and dexamethasone of labeled cyclic adenosine 3,5-monophosphate accumulation by white fat cells. J.biol.Chem. 245: 1101-1107 (1970)

Robinson, J., E.A. Newsholme: Glycerokinase activities in rat heart and adipose tissue. Biochem.J. 104: 2C-4C (1967)

Sutherland, E.W., T.W. Rall: Fractionation and characterization of a cyclic adenine ribonucleotide formed by tissue particles. J.biol.Chem. 232: 1077-1091 (1958)

Umbreit, W.W., R.H. Burris, S.F. Staufer: Manometric Techniques, 4th ed. Burgess Publishing Co., Minneapolis 1964, p. 132

Wick, A.N., D.R. Drury, H.I. Nakada, J.S. Wolfe: Localization of the primary metabolic block produced by 2-deoxyglucose. J.biol.Chem. 224: 963-969 (1957)

Requests for reprints should be addressed to: Dr. Emilio Herrera, Cátedra de Fisiología General, Facultad de Biología, Universidad de Barcelona, Barcelona-7 (Spain)